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Evaluation of different solid-phase traps for automated collection and clean-up in the analysis of multiple pesticides in fruits and vegetables after supercritical fluid extraction

Steven J. Lehotay[a,*], Antonio Valverde-García[b]

[a]US Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

[b]University of Almería, Pesticide Residue Research Group, Faculty of Sciences, 04071 Almería, Spain

Abstract

This study was designed to determine which combination of sorbent-trap and elution solvent provided the most efficient automated method of collection in supercritical fluid extraction (SFE), elution of analytes, and clean-up of orange, sweet potato and green bean extracts for analysis of 56 diverse pesticides using GC–ion-trap MS. The solid-phase traps evaluated consisted of octyldecylsilane (ODS), diol, Tenax and Porapak-Q, and the elution solvents compared were acetone, ethyl acetate, acetonitrile and methanol. SFE collection by bubbling into each organic solvent was also compared. Recoveries, elution volumes, limits of detection and clean-up aspects were determined for each combination of commodity, trap and solvent tested. High trapping efficiencies were achieved in each case, and acetone usually eluted the pesticides in the least volume (<1 ml) from the traps. The few matrix components that interfered in GC–ion-trap MS continued to interfere in all trap/solvent pairs, and limits of detection were independent of trap/solvent combination. The use of the ODS trap and acetone elution solvent gave the most consistently high recoveries of the traps and solvents tested.

Keywords: Fruits; Vegetables; Food analysis; Multiresidue analysis; Sample preparation; Supercritical fluid extraction; Solid-phase extraction; Pesticides

1. Introduction

Many regulatory, industrial and research laboratories around the world routinely analyze fruits and vegetables for multiple pesticide residues. Supercritical fluid extraction (SFE) followed by gas chromatography–ion-trap mass spectrometric detection (GC–ITD) is a promising approach [1–3] to supplant liquid extraction methodologies [4–8] in this application. Initial studies of SFE for multiresidue analysis used Hydromatrix, a pelletized diatomaceous earth material, to control water in moist samples, but that drying agent showed the inability to extract methamidophos [9,1]. However, Valverde-García et al. demonstrated that the use of a magnesium sulfate drying agent gave high recoveries of the problematic pesticide as well as other diverse pesticides [10,11].

SFE has advantages of increased automation, greater selectivity, reduced sample preparation time, lower operating costs and much less waste generated versus common extraction methods requiring organic solvents. The use of GC–ITD also saves time, effort and money in analysis due to its ability to quantify and confirm a variety of analytes at trace concentrations in a complex matrix with a single injection [12]. The most common analytical approach

*Corresponding author.

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currently is to perform analysis with selective GC detectors (flame photometric or nitrogen–phosphorus for organophosphorus pesticides, and electrolytic conductivity or electron-capture for organochlorine pesticides) and confirm the presence of residues separately [4]. The combination of SFE and GC–ITD creates a powerful analytical method with little manual labor [1]. In traditional approaches, despite the use of sensitive and selective detectors, much of the time, effort and cost stems from solvent concentration and extract clean-up steps. SFE conveniently concentrates the extracts during collection, and extracts are often analyzed with no further steps.

Off-line collection in SFE can be performed by introducing the extract into a liquid [13–19], a solid sorbent [18–24], or simply an empty vial [25,26]. Many analytes are stable enough for the latter approach, but this technique is not popular. Unfortunately, some SFE instruments force the operator into a particular collection method, but the most advantageous approach depends on the application. For example, a volatile compound may pass through an inert solid-phase trap, yet remain solubilized in a liquid trap, while another volatile component may require cryogenic collection on a bonded-phase sorbent. Another approach is to collect/clean-up the analyte with a solid sorbent on-line while the fluid remains under supercritical conditions [27–29]. The on-line collection approach has been useful in separating fat from analytes, but it is not easily automated and further reduces sample size because the sorbent takes up volume potentially occupied by sample in the SFE vessel.

The most generally advantageous approach is collection on solid-phase traps. Bonded-phase traps have high collection efficiencies for diverse chemicals, and their use allows for a simple automated clean-up of extracts during elution. In the multiresidue application of SFE with diverse pesticides in a single procedure, the high selectivity of SFE cannot be fully realized because conditions are designed to extract the most difficult analyte at the expense of potential gains in selectivity for analytes more easily extracted. In this situation, an off-line clean-up may remove or reduce analytical interferants.

The main objective of this study was to evaluate automated collection, elution and clean-up of different solid-phase trap and solvent combinations to determine which pair provides the most efficient trapping, elution in the smallest volume, and cleanest extracts for multiresidue GC–ITD analysis of various types of produce. Solid-phase materials used in both gas and liquid chromatography were chosen for study due to the unique exposure of the SFE trap to alternating flows of gas and liquid. In the study, the solid-phase traps consisted of: (1) octyldecylsilane (ODS), which is commonly used in reversed-phase HPLC; (2) silica bonded with diol, which is commonly used in normal-phase HPLC; (3) Tenax, a useful material in environmental air sampling applications; and (4) Porapak-Q, a mid-polarity polymer useful as a GC stationary phase. Glass beads, florisil, alumina, silica and charcoal, although useful for particular classes of pesticides, were found to be inadequate for trapping diverse pesticides [1,3]. The elution solvents tested consisted of acetone, ethyl acetate (EtOAc), acetonitrile (MeCN) and methanol (MeOH) which are used in traditional extraction methods [4–8]. The commodities of orange, sweet potato and green bean were chosen to provide a variety of matrix components for investigation. In the study, the measurement of pesticide recoveries provided a means to determine collection efficiencies of each trap; pesticide elution volumes were measured by collecting elution fractions for each trap/solvent pair; limits of detection (LODs) in GC–ITD were calculated for each combination of commodity, trap, solvent and pesticide to provide a measure of the cleanliness of the extracts.

2. Experimental

2.1. Samples

Oranges, sweet potatoes and green beans were purchased at a local supermarket. We peeled the oranges, removed stems from the green beans and washed the sweet potatoes with water. The samples were chopped into small pieces with a knife, and 100 g portions were stored in closed containers in a freezer. Portions of drying agent, consisting of 100 g of $MgSO_4$, monohydrate (Aldrich, Milwaukee, WI, USA) plus 50 g Hydromatrix (Varian, Harbor City, CA, USA), were stored in a refrigerator. The 100 g

frozen samples were mixed with the 150 g drying agent using a Waring-type blender with a small amount of dry ice (≈10 g) for ≈2 min until the samples appeared homogeneous (a spatula was used periodically to scrape surfaces). The samples were stored in the freezer. Subsamples of 5 g (2 g commodity) were loaded into 7 ml extraction thimbles while still frozen. Whatman GF/F filter paper (Maidstone, UK) was cut into 1 cm diameter disks with a cork borer, and disks were placed at each end of the thimble to keep particles away from the seals. The 0.7 μm filter paper pore size also served to help keep $MgSO_4$ particles from affecting the restrictor. Samples were fortified at the bottom of the thimbles (flow was upward) with 10 μl of a spiking solution in acetone containing 56 pesticides listed in Table 1. The spike concentrations, per 2 g sample, are also listed in Table 1 along with the pesticide classification, quantitation masses, average LODs and retention time (t_r) for each pesticide.

2.2. Chemicals

The solid-phase traps investigated in this study consisted of ODS, diol, Tenax and Porapak-Q. All sorbents, 40 μm particle size contained in 1 ml traps, were obtained from Hewlett-Packard (Little Falls, DE, USA). Acetone, MeCN, EtOAc and MeOH used for elution of the traps were HPLC-grade quality or better. Gas cylinders used for SFE contained SFE grade (99.999% purity) and bone-dry grade carbon dioxide from Potomac Airgas (Hyattesville, MD, USA) with eductor tubes. Zero-grade helium, passed through an in-line water/O_2 trap, was the GC carrier gas. The pesticide standards used in this study were typically 99% or higher purity obtained from the US Environmental Protection Agency (Beltsville, MD, USA, or Research Park, NC, USA). Pesticide stock solutions of ≈1000–10 000 μg/ml in acetone were prepared, and a mixture of 56 pesticides was prepared in acetone from the stock solutions. The pesticides were grouped into concentrations of 20 μg/ml (35 pesticides), 50 μg/ml (12 pesticides), 100 μg/ml (8 pesticides) and 500 μg/ml (acephate) which was devised to relate to the LOD (Table 1). An internal standard solution of 100 μg/ml of [$^2H_{10}$]anthracene and [$^2H_{12}$]chrysene (Cambridge Isotope Laboratories, Woburn, MA, USA) was prepared in acetone, and 10 μl was added to all extracts to give an equivalent of 0.5 μg/g per 2 g sample.

2.3. Extraction

A Hewlett-Packard 7680T supercritical fluid extractor was used for SFE. Extraction conditions were 350 bar and 50°C (CO_2 density=0.90 g/ml), 2 min equilibration time, 20.3 min dynamic time at 2 ml/min (6.0 thimble volumes of CO_2), and 50°C restrictor. Collection with the solid-phase traps was performed at 10°C. Elution was with 1.5 ml (or 3 fractions of 0.5 ml) of solvent at 2 ml/min and 30°C. The trap was rinsed with 4 ml of additional solvent at 30°C and 2 ml/min before the next extraction. In experiments involving collection into the different solvents, a trap (10°C during collection) was used that positioned one end of a 60 cm, ≈0.5 mm I.D., stainless steel tube at the restrictor. The other end was submerged in 5–10 ml of solvent at room temperature in a 40 ml vial (capped with a pierced septum). After SFE, the restrictor and tubing was rinsed with 2 ml of solvent at 2 ml/min and 30°C. The extracts were quantitatively transferred into graduated centrifuge tubes and adjusted to 1.5 ml by gentle solvent evaporation under N_2 for analysis.

The design of the trapping/elution experiments is presented in Table 2. Each SFE sequence consisted of 6 samples with 1 commodity, 1 trap and 2 elution solvents. The first extract was a blank with 1.5 ml elution volume. The blank was used to assess clean-up aspects of the trap/solvent combination, and to serve as a realistic matrix for calibration standards. For spike-1, 3 elution fractions of 0.5 ml each was collected to determine elution volumes of pesticides and matrix components, and spike-2 (1.5 ml elution volume) was used to determine recoveries. The next 3 samples were a replication of the first 3, but using a different solvent. The SFE sequence was repeated with 2 different solvents with the same commodity and trap, and the 20 vials from the 2 SFE sequences formed an analytical sequence. The 2 SFE sequences were repeated with the same commodity and a different trap until all 4 traps were used. Then the entire set of experiments was repeated with a different commodity.

Table 1
Typical retention times (t_r), type of pesticide, quantitation masses, average limits of detection (LOD) and spiking level of each pesticide added to the 2 g sample (internal standards were added after SFE)

Pesticide	Type	t_r (min)	Quantitation masses	LOD (ng/g)	Spike (μg/g)
Dichlorvos	OP	6:51	185	6	0.1
Methamidophos	OP	7:06	141	98	0.5
Mevinphos	OP	9:58	192	9	0.1
Acephate	OP	10:17	136	300	2.5
Tetrahydrophthalimide	Other	11:08	151 (79)[a]	41	0.25
Pentachlorobenzene	OC	11:31	248–252	2	0.1
o-Phenylphenol	Other	11:33	169+170	6	0.25
Omethoate	OP	13:10	156	110	0.5
Propoxur	Other	13:19	152	7	0.1
Diphenylamine	Other	13:40	168+169	2	0.1
Chlorpropham	Other	14:18	127+213	6	0.1
Trifluralin	Other	14:25	264+306	0.6	0.1
Phorate	OP	15:01	75+260 (260)[a]	64	0.1
Hexachlorobenzene	OC	15:17	282–288	5	0.1
Dicloran	OC	15:44	176+206 (206)[a]	10	0.25
Dimethoate	OP	15:49	87+93 (87)[a]	22	0.25
Carbofuran	Other	16:00	164	13	0.1
Atrazine	Other	16:16	200+215	6	0.1
Quintozene	OP	16:18	293–299	8	0.1
Lindane	OC	16:31	181+183	40	0.25
Terbufos	OP	16:43	231	3	0.1
Diazinon	OP	17:02	304	2	0.1
Chorothalonil	OC	17:13	266	24	0.1
Disulfoton	OP	17:24	88+274	8	0.25
Phosphamidon	OP	18:46	264	23	0.25
Vinclozolin	Other	19:02	212+285	9	0.1
Parathion-methyl	OP	19:07	263	6	0.1
Carbaryl	Other	19:24	115+144	8	0.1
Malathion	OP	20:32	173	10	0.1
Chlorpyrifos	OP	20:45	314+316 (316)[a]	9	0.1
Aldrin	OC	20:47	263+293 (293)[a]	16	0.1
Dacthal	OC	20:55	299+301+303	3	0.1
Parathion	OP	21:04	291	8	0.1
Dicofol	OC	21:21	250	19	0.1
Captan	Other	22:45	79	69	0.5
Methidathion	OP	22:16	145	11	0.1
Disulfoton sulfone	OP	23:42	213	14	0.1
Endosulfan I	OC	23:49	337+339	27	0.1
Fenamiphos	OP	24:19	303	50	0.5
p,p'-DDE	OC	24:42	316+318	3	0.1
Myclobutanil	Other	25:03	179	42	0.5
Endosulfan II	OC	26:07	337–341	29	0.1
Ethion	OP	26:22	231	4	0.1
o,p'-DDT	OC	26:24	235	14	0.1
Propargite	Other	28:27	135+350	41	0.25
Iprodione	Other	29:24	314+316	25	0.25
Phosmet	OP	29:35	160	7	0.25
Methoxychlor	OC	29:56	227	14	0.1
Phosalone	OP	31:02	182+367	10	0.1
Azinphos-methyl	OP	31:12	77+132+160	47	0.5

Table 1 (*continued*)

Pesticide	Type	t_r (min)	Quantitation masses	LOD (ng/g)	Spike (μg/g)
cis-Permethrin	OC	34:08	183	8	0.1
trans-Permethrin	OC	34:33	183	9	0.1
Cyfluthrin	OC	36:02	206	214	0.5
Cypermethrin	OC	37:16	163+181	113	0.5
Fenvalerate	OC	41:30	125+225+419	62	0.25
Esfenvalerate	OC	42:43	125+225+419	69	0.25
[$^2H_{10}$]Anthracene	I.S.	17:16	188	8	0.5
[$^2H_{12}$]Chrysene	I.S.	29:45	240	23	0.5

[a]Quantitation mass for sweet potato.
I.S.=internal standard; OP=organophosphorus; OC=organochlorine.

2.4. Analysis

Analysis was performed using a Finnigan ITS40 (San Jose, CA, USA) gas chromatograph–ion-trap mass spectrometer. Operating conditions for GC–ITD were: DB-5ms (J&W Scientific, Folsom, CA, USA), 30 m×0.25 mm I.D., 0.25 μm film thickness, capillary column, 5 m phenyl-methyl deactivated guard column, 0.25 mm I.D. (Restek, Bellefonte, PA, USA), 1 μl injection volume, Model 1093 (Varian, Walnut Creek, CA, USA) septum programmable injector (SPI); 50°C injection port for 6 s followed by ramping to 260°C at 10°C/min; 10 p.s.i.g. He column head pressure (34 cm/s at 50°C) (1 p.s.i.= 6894.76 Pa); 50°C initial oven temperature for 1 min, ramped to 130°C at 10°C/min, then to 260°C at 6°C/min, and held at 260°C until a 45 min total time had elapsed; 260°C transfer line temperature; and 220°C ion-trap manifold temperature. A modification of the SPI was made so that the septum purge flow

Table 2
Outline of experiments in the study[a]

I. Commodity=orange
- A. Sorbent=ODS
  - 1. Elution solvent=acetone
    - a. Blank (1.5 ml elution volume)
    - b. Spike-1
      - (1) 0–0.5 ml elution fraction
      - (2) 0.5–1 ml elution fraction
      - (3) 1–1.5 ml elution fraction
    - c. Spike-2 (1.5 ml elution volume)
  - 2. Elution solvent=acetonitrile
    - a. Blank (1.5 ml elution volume)
    - b. Spike-1
      - (1) 0–0.5 ml elution fraction
      - (2) 0.5–1 ml elution fraction
      - (3) 1–1.5 ml elution fraction
    - c. Spike-2 (1.5 ml elution volume)
  - 3. Elution solvent=ethyl acetate
    - a. Blank (1.5 ml elution volume)
    - b. Spike-1
      - (1) 0–0.5 ml elution fraction
      - (2) 0.5–1 ml elution fraction
      - (3) 1–1.5 ml elution fraction
    - c. Spike-2 (1.5 ml elution volume)
  - 4. Elution solvent=methanol
    - a. Blank (1.5 ml elution volume)
    - b. Spike-1
      - (1) 0–0.5 ml elution fraction
      - (2) 0.5–1 ml elution fraction
      - (3) 1–1.5 ml elution fraction
    - c. Spike-2 (1.5 ml elution volume)
- B. Sorbent=Diol C. Sorbent=Tenax D. Sorbent=Porapak-Q
  Repeated 1–4 with each sorbent

II. Commodity=Sweet Potato
Repeated A–D with each commodity

III. Commodity=Green Bean

[a]An SFE sequence consisted of 6 extractions with 1 trap and 2 elution solvents (resulting in 10 vials), and a GC–ITD sequence consisted of 2 SFE sequences (20 vials) with 1 trap and 4 elution solvents (blank extracts were spiked to serve as calibration standards).

was turned off during injection and on again 5 min later. The septum and injection liner were changed, and ≈30 cm of the guard column was removed after every sequence of 20 samples. Typical ITD operating conditions were: electron impact mode, 12 μA filament current, 1500 V electron multiplier (K&M, West Springfield, MA, USA), and automatic gain control at 20 000. The collection range was 70–370 u from 6.5 min to 40 min, and 110–420 u from 40–45 min for the analysis of the pesticides.

Quantitation masses were selected for each pesticide based on achieving the highest signal-to-noise ratio in the matrix. Blank extracts were initially injected to determine possible matrix interferences, then they were fortified with the spiking solution to serve as calibration standards in matrix. Relatively few interferences occurred, and Table 1 indicates the changes in quantitation masses made to minimize the effect of sweet potato interferences. The GC–ITD utilized Magnum Version 2.4 software for data collection; peak heights were used in all calculations. Recoveries were calculated by dividing the pesticide signal versus [$^2H_{12}$]chrysene signal of a spiked sample by the average of the signal versus [$^2H_{12}$]chrysene responses of the 4 calibration standards (concentrations=25%, 50%, 100% and 200% of spiking level), which were normalized to the 100% standard. Recoveries of pesticides affected by solvent were calculated versus the standard in the same solvent (Section 3.1). LOD (assuming 100% recovery) for each pesticide was determined in each standard by multiplying the known concentrations by 3 and dividing by the signal/noise ratio (as reported by the GC–ITD software).

3. Results and discussion

3.1. Effect of different solvents in analysis

An initial experiment was performed to determine if pesticides in the different solvents (acetone, MeCN, EtOAc and MeOH) gave different analytical responses or notable effects in GC–ITD. Blank SFE extracts for each commodity/trap/solvent combination were spiked for use as calibration standards. The initial GC injection port and oven temperature of 50°C was used so that extracts were injected on-column as liquids. For orange and sweet potato, no significant differences were observed in pesticide responses with the different solvents. However, green bean extracts in MeCN gave disproportionally higher responses for the most polar pesticides in the mixture, and EtOAc gave lower responses for those pesticides, as shown in Table 3. The apparent reason for the effect is the higher amount of water in the green bean extracts than with the other commodities. Table 3 also lists the solubilities of the pesticides in water to show a correlation of the effect versus the solubilities. In the case of EtOAc, water is not miscible with the solvent, and, in fact, a small bead of a turbid second liquid-phase was sometimes present in the green bean extracts in EtOAc. A partitioning of the more polar pesticides into the aqueous phase occurred, thereby reducing the response of those pesticides in the EtOAc. The case of MeCN is not as easily explained because MeOH and acetone are also miscible with water. Note that MeOH also gave enhanced response versus acetone for the most polar pesticides, methamidophos and omethoate. The mechanism of this effect is unknown, but it is likely to be related to a more efficient on-column injection of the MeCN extracts due to the higher boiling point of MeCN (81.6°C), and to a lesser extent MeOH (64.7°C), than acetone (56.2°C). Perhaps, the presence of water in the different solvents gave different levels of interaction of active surface sites in the injection liner with the polar pesticides.

3.2. SFE collection by bubbling into solvents

Some SFE instruments do not allow collection with solid-phase traps, and for purposes of comparison, a solvent trapping experiment was conducted. There were no significant differences in pesticide recoveries when using acetone, MeCN, EtOAc or MeOH for collection. The different solvents did evaporate during collection at different rates based on their volatility, but none of the collection vials went to dryness during extraction. An interesting aspect of the collection approach was that the solvent evaporation rate was lower in this experiment compared to studies using a linear restrictor [10,11]. The reason for this difference was the separation of the variable restrictor from the

Table 3
Effect of solvent and pesticide solubility in water on the average response (peak height vs. [$^2H_{12}$]chrysene peak height), $n=4$, of standards in green bean extracts for selected pesticides normalized to the average response for acetone

Pesticide	Solubility in water[a]	Normalized response			
		MeCN	EtOAc	MeOH	Acetone
Dichlorvos	10 g/l	2.80	0.76	0.88	1.00
Methamidophos	2000 g/l	2.72	0.74	1.22	1.00
Mevinphos	600 g/l	2.21	0.91	0.97	1.00
Acephate	650 g/l	1.95	0.30	1.05	1.00
Tetrahydrophthalimide		1.30	0.61	0.90	1.00
Pentachlorobenzene	insoluble	1.10	1.05	1.04	1.00
o-Phenylphenol	700 mg/l	1.22	1.02	0.92	1.00
Omethoate	miscible	3.66	0.78	2.17	1.00
Propoxur	2 g/l	1.25	0.83	0.81	1.00
Diphenylamine		1.04	1.05	0.95	1.00
Trifluralin	0.3 mg/l	1.04	0.93	0.90	1.00
Dicloran	7 mg/l	1.03	0.96	0.91	1.00
Dimethoate	25 g/l	1.46	0.95	0.90	1.00
Atrazine	33 mg/l	1.18	0.99	1.02	1.00
Lindane	7 mg/l	0.98	0.97	0.96	1.00
Diazinon	40 mg/l	1.10	0.95	1.00	1.00
Vinclozolin	3 mg/l	0.99	1.00	1.06	1.00
Malathion	145 mg/l	1.05	0.91	0.93	1.00
Chlorpyrifos	1 mg/l	0.98	0.97	1.07	1.00
Propargite	0.6 mg/l	1.02	0.92	1.04	1.00
Iprodione	14 mg/l	0.99	0.90	0.95	1.00
Fenvalerate	<1 mg/l	0.97	0.95	1.01	1.00

[a]Data mostly from the Pesticide Properties Database (http://ncsr.arsusda.gov/ppdb3).

solvent by wide-bore tubing in this experiment as opposed to essentially immersing the restrictor into the solvent with linear restrictors (capillary tubing). With the variable restrictor, higher dynamic flow-rate can be achieved without affecting solvent trapping, and the restrictor and tubing can be rinsed more easily after SFE.

Table 4 lists the recoveries of the pesticides when collection was performed by bubbling the extracts into different solvents. The restrictor/tubing rinse for sweet potato was analyzed separately from the solvent in the collection vial, and the results (presented as % of total recovery in each fraction) are also presented in Table 4. A clear trend of % dissolved in the collection solvent (as opposed to % condensed in the tubing) versus pesticide volatility occurred. A higher percentage of the most volatile pesticides, dichlorvos (64%), mevinphos (28%), pentachlorobenzene (40%) and hexachlorobenzene (18%), passed through the tubing and dissolved in the trapping solvent. The other pesticides condensed mostly in the restrictor and/or tubing leading to the solvent collection vial, and less than 15% of the total for any other pesticide was collected in the solvent trap. This data shows which pesticides can be collected through simple condensation onto a surface [26], and which require more careful trapping. It also reinforces the fact that the restrictor should be flushed with solvent after extraction to achieve higher recoveries [30,31].

The solvent trapping experiments gave more reproducible recoveries (16% average R.S.D.) than trapping on sorbents (29% average R.S.D.) due to the greater variation in results between different sorbent trap/elution solvent combinations than in the case of trapping in different solvents. The reproducibility of recoveries improved with ODS/acetone, for example, which gave average an R.S.D. of 15%.

A very slight increase in overall recovery versus t_r (decreasing volatility) for the pesticides was observed for SFE trapping in solvents. Average recovery of pesticides with $t_r<20$ min (before malath-

Table 4
The effect of rinsing the restrictor and tubing with solvent after extraction, and average SFE %recoveries (%R.S.D.) of the pesticides with collection in a solvent trap versus collection with solid-phase traps

Pesticide	Solvent trapping		Solvent trapping %recovery (n=12)	Solid-phase trapping %recovery (n=42)
	% in tubing	% in solvent		
Dichlorvos	36	64	70 (24)	78 (41)
Methamidophos	100	ND	48 (22)	56 (52)
Mevinphos	72	28	68 (9)	76 (34)
Acephate	89	11	70 (21)	54 (65)
Tetrahydrophthalimide	85	15	81 (7)	88 (42)
Pentachlorobenzene	60	40	79 (19)	76 (16)
o-Phenylphenol	87	13	71 (6)	74 (28)
Omethoate	72	18	54 (24)	57 (60)
Propoxur	88	12	68 (8)	79 (27)
Diphenylamine	89	11	69 (8)	72 (23)
Chlorpropham	91	9	72 (8)	73 (26)
Trifluralin	90	10	72 (11)	80 (18)
Phorate	95	5	75 (13)	68 (28)
Hexachlorobenzene	72	18	69 (9)	75 (18)
Dicloran	94	6	73 (17)	74 (22)
Dimethoate	88	12	69 (9)	79 (28)
Carbofuran	93	7	70 (10)	77 (27)
Atrazine	91	9	77 (14)	74 (30)
Quintozene	92	8	70 (9)	77 (17)
Lindane	94	6	69 (9)	78 (18)
Terbufos	91	9	68 (20)	76 (25)
Diazinon	91	9	76 (10)	78 (18)
Chorothalonil	100	ND	52 (45)	67 (45)
Disulfoton	94	6	57 (21)	65 (26)
Phosphamidon	88	12	62 (20)	73 (35)
Vinclozolin	95	5	72 (12)	82 (28)
Parathion-methyl	95	5	72 (12)	74 (26)
Carbaryl	95	5	81 (10)	80 (27)
Malathion	92	8	71 (13)	81 (24)
Chlorpyrifos	95	5	69 (26)	80 (26)
Aldrin	100	ND	80 (13)	71 (16)
Dacthal	92	8	78 (12)	80 (12)
Parathion	96	4	76 (14)	77 (24)
Dicofol	95	5	77 (15)	78 (21)
Captan	96	4	62 (57)	44 (73)
Methidathion	96	4	84 (10)	89 (24)
Disulfoton sulfone	95	5	76 (10)	75 (28)
Endosulfan I	100	ND	66 (16)	74 (23)
Fenamiphos	97	3	81 (28)	65 (36)
p,p'-DDE	93	7	78 (14)	78 (15)
Myclobutanil	97	3	69 (29)	60 (40)
Endosulfan II	100	ND	74 (20)	74 (19)
Ethion	92	8	74 (17)	77 (26)
o,p'-DDT	95	5	72 (14)	73 (31)
Propargite	100	ND	76 (15)	77 (24)
Iprodione	96	4	73 (10)	77 (26)
Phosmet	95	5	80 (12)	82 (31)

Table 4 (*continued*)

Pesticide	Solvent trapping		Solvent trapping %recovery (n=12)	Solid-phase trapping %recovery (n=42)
	% in tubing	% in solvent		
Methoxychlor	92	8	77 (14)	79 (42)
Phosalone	96	4	71 (12)	77 (24)
Azinphos-methyl	97	3	80 (9)	83 (42)
cis-Permethrin	95	5	79 (14)	81 (16)
trans-Permethrin	95	5	74 (16)	81 (16)
Cyfluthrin	100	ND	60 (29)	80 (24)
Cypermethrin	94	6	102 (23)	85 (25)
Fenvalerate	91	9	87 (23)	81 (22)
Esfenvalerate	96	4	76 (15)	84 (26)

ND=not detected.

ion) was 72±4%, while average recovery of pesticides with t_r>20 min was 77±7%. (Methamidophos, acephate and omethoate were not included in these calculations due to incomplete extraction, and disulfoton, chlorothalonil and captan were not included due to partial degradation.) For comparison, the average recoveries of the same pesticides with solid-phase traps was 77±4% for pesticides with t_r<20 min and 78±6% for pesticides with greater t_r. This trend shows the slight degree of pesticide losses due to volatilization in SFE collection with solvents versus trapping on solid sorbents. Trapping with solvents at lower temperature should decrease losses due to volatilization. However, the losses may have occurred during the solvent evaporation step before analysis rather than during SFE collection, which is a step that is not needed when trapping on sorbents.

3.3. SFE collection with solid-phase traps

Despite recoveries <100% and average R.S.D. of 29% shown in Table 4, trapping efficiency was believed to be nearly 100% with each solid-phase trap tested in this study. Previous studies with ODS trapping gave systematically higher recoveries [1,32]. Reduced recovery during trapping would lead to differences in results based on chemical nature of the pesticides [32], such as volatility in the case of trapping with solvents. In situations when an individual pesticide recovery was 10% below the mean recovery of all pesticides, the discrepancy was the result of pesticide degradation (captan, disulfoton, chlorothalonil), incomplete extraction (methamidophos, acephate, omethoate), incomplete trap elution (Section 3.4) or analytical error. The complex nature of the different commodities, water content and mixture of Hydromatrix and $MgSO_4$ was also likely to affect SFE.

3.4. Elution

For most sensitive analysis, the amount of solvent needed to desorb the pesticides from the trap should be minimized. To determine elution volumes for the pesticides, three 0.5 ml elution fractions were collected in each commodity/trap/solvent combination for a spiked sample. Nearly all pesticides were detected within 1 ml volume in each combination except for Tenax using MeOH and MeCN for elution. A trend of lower recoveries were obtained with MeOH in nearly all combinations, and the effect was more pronounced in the case of nonpolar pesticides. Hexachlorobenzene, the most nonpolar analyte, was the last pesticide to elute from the trap in nearly every case. Fig. 1 gives the elution profiles of hexachlorobenzene after SFE of orange for each trap/solvent combination. The more polar pesticides were not usually detected in the third fraction, but the reduced recoveries with MeOH elution indicated incomplete elution within 1.5 ml.

Table 5 gives more detailed recovery results for representative pesticides using the different traps and solvents. The highest recovery for each pesticide within a set of trap and solvents is in bold, and the lowest recovery is italicized. Overall, acetone gave

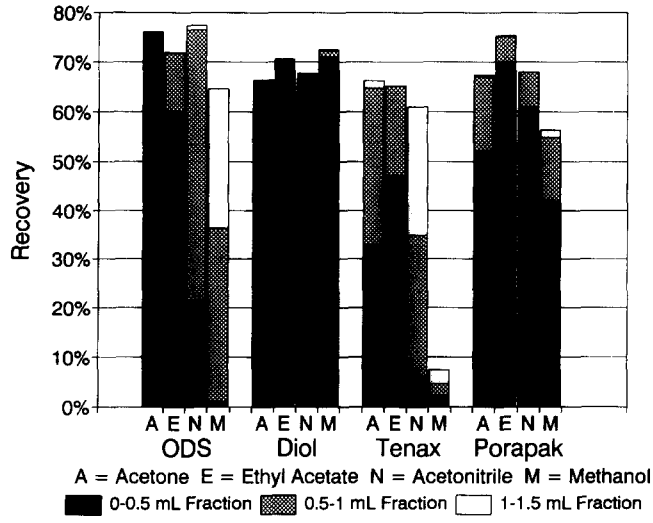

Fig. 1. SFE recoveries of hexachlorobenzene from orange, divided into three 0.5 ml elution fractions, versus the different solid-phase trap and elution solvent combinations.

the highest recoveries for the most pesticides, and MeOH was the lowest in most cases. MeOH was the 'weakest' elution solvent, and, in general, the order of solvent 'strength' for multiresidue extraction can be ranked as: acetone>EtOAc=MeCN>MeOH. This trend also generally occurs in the different liquid-based multiresidue extraction methods [33], and illustrates why MeOH is not commonly used for extraction in multiresidue methods [4–8]. Based on elution results, the overall pesticide 'retention strength' of the different sorbents can be ranked as: Tenax>Porak-Q>diol=ODS.

Curious recovery results are shaded for the combination of Porapak-Q and methanol. Several pesticides were not detected, presumably because recoveries were lower than could be detected. For those pesticides that were detected, many gave anomalously high recoveries, even when using only calibration standard prepared in blank Porapak/MeOH extracts. Some pesticides were unaffected by the trap/solvent pair for unknown reasons. It is known that MeOH will dissolve the coating for many GC stationary phases, and apparently this was the case for Porapak-Q. No unusual compounds appeared in the chromatograms of Porapak/MeOH extracts, but GC–ITD failed on two occasions during analysis of Porapak/MeOH extracts. MeOH was also ineffective in combination with Tenax, but results were consistently low, not abnormally high.

Fig. 2 is a graph of the number of pesticides with average recovery ≥70% and %R.S.D.≤30% for each sorbent trap/elution solvent combination (n=3, one determination per commodity). The combination of ODS trapping and elution with acetone gave the most pesticides (51 out of 56) meeting the recovery criteria for the 3 commodities. This combination of a strong elution solvent with a relatively weak sorbent made the ODS/acetone pair the most consistent of those tested. The only pesticides that did not meet these criteria for ODS/acetone were: omethoate (58% recovery, 71% R.S.D.), captan (36%, 57%), myclobutanil (65%, 36%), azinphos-methyl (86%, 43%), and cypermethrin (104%, 33%). Omethoate was incompletely extracted by SFE (and gave poor GC–ITD peak shape), but azinphos-methyl and cypermethrin usually gave more reproducible results. Captan, which was prone to degradation, and myclobutanil, which gave poor GC–ITD peak shape, did not meet the arbitrary criteria for any trap/solvent pair.

If the acceptance criteria are set to be recovery ≥80% and R.S.D.≥20%, 39 of the 56 pesticides give acceptable results for ODS/acetone, while the next best trap/solvent combination is Porapak/

Table 5
Average %recoveries, $n=3$, of representative pesticides with the different solid-phase traps and elution solvents (**high** and *low* recoveries designated within a set of solvents for each trap and pesticide)

Pesticide	ODS				Diol				Tenax				Porapak			
	A	E	N	M	A	E	N	M	A	E	N	M	A	E	N	M
OP pesticides																
Dichlorvos	**84**	*62*	80	68	**78**	62	65	*50*	81	*46*	**84**	75	102	*82*	**124**	108
Methamidophos	**72**	49	64	*38*	57	42	**70**	*40*	**102**	46	86	*24*	**59**	*18*	49	293
Mevinphos	**102**	68	*66*	79	**82**	*56*	62	57	79	64	**110**	*59*	84	74	**96**	*70*
Dimethoate	**95**	80	83	*71*	79	80	**100**	*66*	91	69	**93**	*53*	73	**89**	71	*19*
Diazinon	82	79	**90**	*71*	**81**	74	75	*69*	**85**	77	83	*62*	*79*	83	**84**	175
Chlorpyrifos	**97**	74	72	*64*	84	**88**	*79*	*79*	77	76	**84**	*34*	*70*	**97**	79	143
Parathion	**86**	78	83	*70*	*62*	**74**	73	64	71	76	**93**	*38*	82	78	**83**	*75*
Methidathion	**98**	84	93	*75*	**123**	87	89	*80*	84	78	**87**	*24*	87	100	*80*	**108**
Ethion	73	80	**86**	*67*	**77**	**77**	74	*65*	**77**	72	76	*43*	81	*77*	87	**92**
Azinphos-methyl	86	67	**96**	*55*	84	69	**110**	*62*	**94**	68	91	*21*	97	**118**	*78*	*78*
OC pesticides																
Hexachlorobenzene	**88**	75	87	*71*	**81**	78	72	*68*	**75**	72	53	*12*	*74*	**77**	76	269
Dicloran	**85**	77	77	*65*	**70**	65	68	*53*	**89**	71	85	*54*	79	74	**88**	*72*
Lindane	**94**	*85*	*85*	93	76	**79**	76	*70*	**72**	69	**72**	*63*	*76*	78	**82**	160
Chlorothalonil	**87**	83	78	*65*	77	**78**	68	*54*	33	**55**	53	*21*	*40*	**145**	54	ND
Dacthal	**86**	*77*	85	78	80	81	*79*	**85**	76	77	**78**	*37*	78	**88**	*76*	205
Dicofol	**93**	85	**93**	*79*	71	**75**	**75**	*62*	**74**	63	67	*18*	*83*	*83*	**88**	168
p,p'-DDE	86	76	**88**	*75*	77	**80**	74	*65*	**78**	**78**	77	*22*	*74*	**83**	78	258
Endosulfan II	**78**	*76*	**78**	**78**	**78**	70	75	*68*	74	*69*	72	**75**	*64*	72	**80**	ND
cis-Permethrin	92	80	**95**	*70*	77	**82**	79	*70*	**88**	83	80	*20*	*78*	83	**85**	170
Fenvalerate	**93**	85	86	*68*	74	**85**	76	*70*	81	74	**92**	*36*	85	*77*	83	**100**
Other pesticides																
Tetrahydrophthalimide	95	*68*	**96**	75	97	86	**116**	*70*	**146**	102	101	*65*	**83**	72	*67*	ND
o-Phenylphenol	**91**	77	82	*72*	**75**	66	73	*63*	74	73	**84**	*58*	77	**85**	**85**	*60*
Diphenylamine	**82**	*70*	75	76	67	**70**	66	*58*	74	68	**75**	*45*	*65*	69	80	**84**
Trifluralin	**92**	84	88	*71*	**80**	79	77	*62*	81	68	**88**	*36*	84	*83*	*90*	181
Carbofuran	**97**	83	87	*77*	**78**	66	67	*55*	72	74	**88**	*53*	85	**94**	*72*	ND
Atrazine	86	*68*	**92**	75	67	63	**69**	*54*	**77**	72	**77**	*57*	77	*66*	79	**95**
Vinclozolin	**89**	*80*	84	*80*	74	**75**	74	*67*	**84**	78	79	*33*	*75*	**86**	**86**	118
Myclobutanil	**65**	42	59	*40*	**72**	68	69	*61*	58	43	**76**	*26*	**69**	54	66	*52*
Propargite	78	*68*	**91**	78	**97**	84	81	*72*	**69**	63	68	*22*	**80**	76	*74*	ND
Iprodione	**96**	80	76	*60*	67	**75**	74	*66*	67	68	**90**	*42*	94	103	70	ND

A=acetone; E=EtOAc; N=MeCN; M=MeOH.

EtOAc with 26 pesticides. The other 12 pesticides for ODS/acetone that did not meet the more stringent acceptance criteria consist of: methamidophos (72% recovery, 21% R.S.D.), phorate (78%, 3%), terbufos (73%, 4%), disulfoton (79%, 15%), chlorpyrifos (97%, 21%), lindane (94%, 30%), aldrin (89%, 26%), endosulfan I (77%, 5%), endosulfan II (78%, 17%), *o,p'*-DDT (72%, 29%), tetrahydrophthalimide (95%, 29%) and diphenylamine (82%, 24%). Phorate, terbufos and disulfoton were consistently low due to partial conversion to sulfones and sulfoxides; tetrahydrophthalimide was variable because it is a metabolite of captan; and chlorpyrifos, lindane and aldrin were affected by matrix interferants in sweet potato (Section 3.5). It is noteworthy that many of the same pesticides that present problems in SFE and GC–ITD often present problems in traditional multiresidue methods [4–8].

3.5. Clean-up

Table 6 charts a subjective description of the

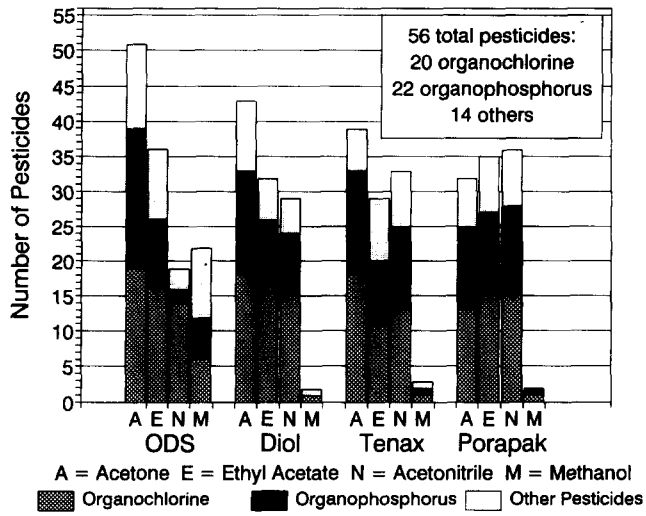

Fig. 2. The number of pesticides giving mean recovery ≥70% with R.S.D. ≤30% for the orange, sweet potato and green bean matrices, divided into the pesticide classes, versus the different solid-phase trap and elution solvent combinations.

appearance of the extracts and denotes if a precipitate was observed. The use of MeCN and MeOH for elution generally provided the least colored extracts, and a colored precipitate was often observed in MeCN and MeOH extracts. The colored materials were soluble in acetone and EtOAc which made extracts in those solvents much darker, but, in general, the lighter colored extracts did not contain less intense matrix peaks than darker colored extracts. None of the solvents sufficiently removed matrix components to make significant differences in affecting analysis of the pesticides, or in extending the number of samples that could be injected before GC maintenance was necessary. Typically, peak shape for omethoate, the most problematic pesticide for analysis, began to broaden after ≈10 injections.

Fig. 3 presents the SFE sweet potato extract collected on ODS and eluted in three 0.5 ml fractions with acetone. The first fraction contained the large majority of matrix components, with a 100% total ion current (TIC) of 33.6×10^6 counts, and the second fraction (100% TIC=13.8×10^6 counts) contained few major peaks at lower t_r, but several peaks with longer t_r appeared. The third fraction (100% TIC=0.3×10^6 counts), was relatively free of matrix components that appeared in the chromatogram. The GC–ITD software with the mass spectral libraries provided was not able to identify the major matrix components. No other peaks appeared in the solvent trapping experiments other than those that appeared in the solid-phase trapping experiments. With other traps and solvents tested, the same matrix peaks

Table 6
Appearance of the SFE extracts in terms of color intensity and presence of precipitate for the different commodity, collection methods, and solvent combinations

Collection method	Orange				Sweet potato				Green bean			
	Acetone	EtOAc	MeCN	MeOH	Acetone	EtOAc	MeCN	MeOH	Acetone	EtOAc	MeCN	MeOH
ODS	x	x	–	–	xxx	xxx	x	x	xx	xx	x	x
Diol	x	x	x	x*	xxx	xxx	xxx*	xx	xx	xx	xx	xx
Tenax	x	x	–	–	xxx	xxx	xxx*	xx	xx	xx	xx	x
Porapak	x	x	–	–	xxx	xxx	xxx*	xx*	xx	x	xx	x
Bubbling	–	–	–*	–	xxx	xxx	xxx	xxx	x	x	x	x

–=colorless; x=very light coloration; xx=light coloration; xxx=moderate coloration; *very small amount of precipitate.

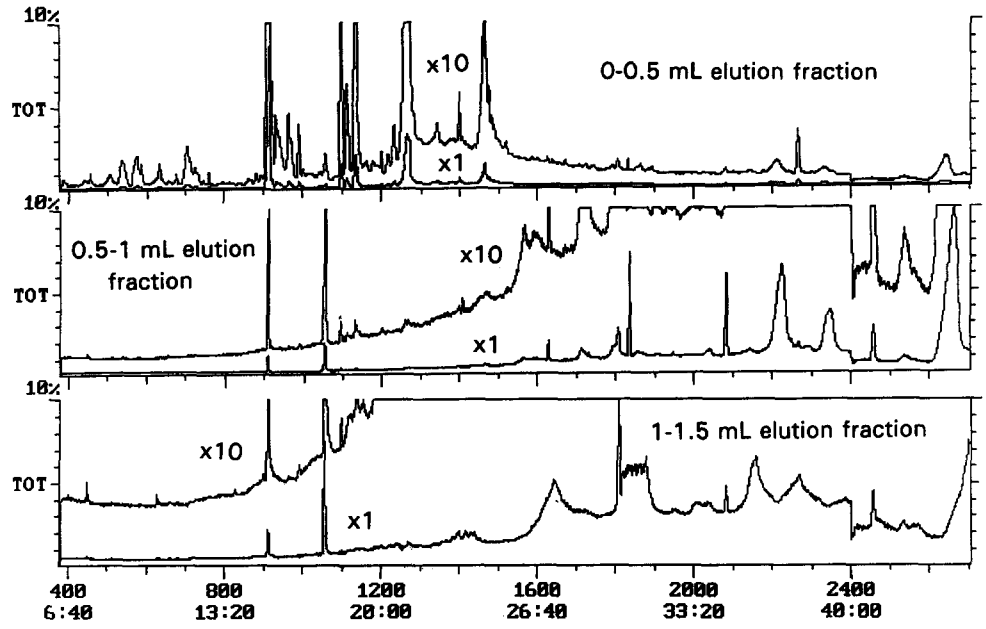

Fig. 3. GC–ITD total ion chromatograms of three 0.5 ml elution fractions with acetone of sweet potato SFE extracts trapped on ODS.

occurred as with ODS/acetone, but the major components were often more pronounced in the second and third fractions. In comparison, the TIC averaged 35, 5 and 1×10^6 counts for fractions 1, 2 and 3, respectively, for the elution of sweet potato extracts from ODS with the other solvents. Unfortunately, the major interferants eluted from the trap with many of the pesticides. The use of water to first rinse matrix components from the traps may have improved clean-up, but this approach would complicate the overall procedure (more time-consuming, larger volumes and need for water removal before analysis). The elution profiles of the pesticides (Fig. 1) in the case of ODS/acetone indicated that 0.5 ml was sufficient for nearly 100% elution of all pesticides, and the later-eluting sweet potato components in Fig. 2 (0.5–1.5 ml fractions) could be avoided by using a 0.5 ml elution volume. Furthermore, LOD would be reduced by a factor of 3 for those pesticides not affected by matrix interferants (compare Table 1 and Table 7) and the GC column and ion-trap would be spared exposure to possible undetected matrix components retained on the trap from the 0.5–1.5 ml acetone fraction.

Table 7 provides an indication of the effect of matrix on the LOD of the pesticides. No significant differences among the matrices were observed for pesticides other than those listed, and significant differences in LOD did not correlate with the trap or solvent used. Decreasing LOD of late-eluting pyrethroid pesticides were due to the reduction of column bleed interferants as the analytical column aged (presumably a result of extended times at high temperature). SFE solvent-based trapping gave similar LOD and were included in the overall matrix averages (Table 1).

All sweet potato extracts contained large matrix components that most severely overlapped with phorate, chlorpyrifos and aldrin. The quantitation masses in these (and other) cases were altered as listed in Table 1 to help overcome the matrix interferants. Fig. 4 is a chromatogram of a sweet potato extract collected on ODS and eluted with acetone showing pesticide peaks at selected ions despite the presence of the large interferants. It was still possible to accurately quantify concentrations, as demonstrated in Tables 3–5, but these pesticides could be confirmed with the method only at very high concentrations. As reflected in Table 7, orange extracts contained no matrix components affecting analysis. Green bean contained many matrix components, but of much lower intensity than sweet

Table 7
Average limits of detection of those pesticides with significant differences in the different commodities

Pesticide	Limit of detection (ng/g)		
	Orange	Sweet Potato	Green Bean
Tetrahydrophthalimide	9	79	34
o-Phenylphenol	2	13	3
Phorate	2	18	5
Hexachlorobenzene	1	14	1
Dicloran	8	178	6
Carbofuran	5	26	8
Quintozene	3	15	6
Lindane	15	66	39
Chlorothalonil	8	48	16
Vinclozolin	4	17	5
Parathion-methyl	3	11	3
Carbaryl	4	15	6
Malathion	2	23	5
Chlorpyrifos	1	24	3
Aldrin	3	31	14
Parathion	3	15	5
Dicofol	6	41	10
Captan	17	153	38
Disulfoton sulfone	4	33	4
Endosulfans	8	54	22
Fenamiphos	44	81	25
o,p'-DDT	5	21	15
Propargite	25	47	50
Iprodione	11	44	21
Methoxychlor	7	17	19
Phosalone	6	10	14
Cyfluthrin[a]	304[a]	200[a]	137[a]
Cypermethrin[a]	122[a]	113[a]	104[a]
Fenvalerates[a]	92[a]	57[a]	48[a]

[a]Decreasing LOD due to aging of column, not matrix.

potato, and only phosalone presented a situation similar to phorate, chlorpyrifos and aldrin in sweet potato. Analytical options for confirmation in this situation could possibly involve the use of: (1) different GC separation conditions; (2) further clean-up; (3) a selective detector; (4) chemical ionization; or (5) MS–MS analysis [34].

4. Conclusions

Several conclusions can be made from this study. Foremost, SFE collection with ODS and elution with acetone gave the most reproducibly high pesticide recoveries. Other traps were equally efficient in SFE collection, but pesticide elution in 1.5 ml from more retentive stationary phases with weaker elution solvents proved to be less reproducible. In general, the strength of pesticide retention in SFE trapping appeared to be: Tenax>Porapak-Q>diol=ODS>solvents>surface trapping. The elution strength of the solvents was: acetone>EtOAc=MeCN>MeOH. MeOH was incompatible as an elution solvent with Porapak-Q, and gave the lowest pesticide recoveries in nearly all cases. Trapping was very efficient for the diverse pesticides with the bonded-phase sorbents studied, and only volatile pesticides gave slightly lower recoveries using SFE collection in solvent traps and pesticide condensation on surfaces. However, SFE collection by bubbling into solvents was not automated, provided no clean-up, required a solvent evaporation step before analysis, and lost a small percentage of volatile pesticides. None of the trap/solvents cleaned-up the matrix sufficiently to

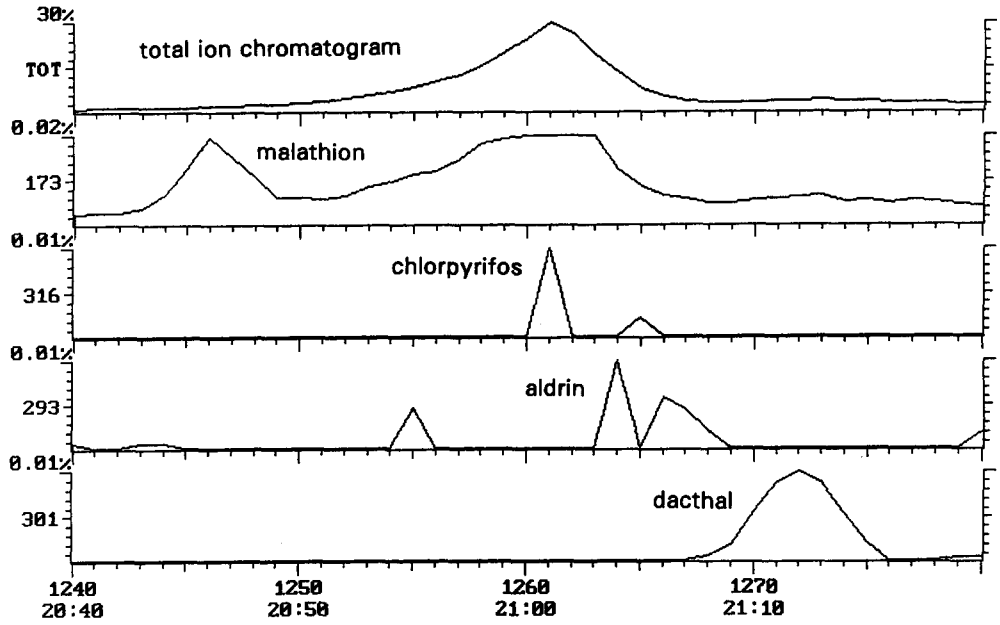

Fig. 4. GC–ITD peaks of malathion, chlorpyrifos, aldrin and dacthal in the presence of a large matrix component of a SFE sweet potato extract trapped on ODS and eluted with acetone.

reduce the effect of interferants when they occurred. The reduced sample size and increased selectivity of SFE versus traditional methods provided the bulk of the sample clean-up for analysis, but collection on solid-phase traps and elution with minimal solvent did remove some matrix components. With the use of fortified matrix blanks as calibration standards, quantitation was not profoundly affected by the presence of large matrix peaks; however, pesticide confirmation was not possible when large matrix peaks overlapped.

Altogether, ≈160 extractions were performed in this study and ≈270 analyses, including solvent trapping experiments and blanks, which generated more than 15 000 data points for all 56 pesticides. Sample throughput was not maximized in this study, but the automated systems generated data very quickly with minimal human effort (organizing and interpreting the data was the limiting factor). In a lab designed for routine analysis, 2 SFE sequences could be run per day which would require approximately 2 h of an operator's time spent preparing samples, loading thimbles and performing routine maintenance, and 1 GC–ITD sequence of 20 samples could be run per day. An organized individual could keep up with the daily extraction and analysis process, including peak integration and instrument maintenance, if detailed interpretation of data and preparation of reports were not required.

Acknowledgments

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References

[1] S.J. Lehotay and K.I. Eller, J. AOAC Int., 78 (1995) 821–830.
[2] S.J. Lehotay, N. Aharonson, E. Pfeil and M.A. Ibrahim, J. AOAC Int., 78 (1995) 831–840.

[3] S.J. Lehotay and M.A. Ibrahim, J. AOAC Int., 78 (1995) 445–452.
[4] M.A. Luke and H.T. Hasumoto, Anal. Meth. Pest. Plant Growth Reg., 15 (1986) 161–200.
[5] A. Andersson and H. Pålsheden, Fresenius J. Anal. Chem., 339 (1991) 365–367.
[6] S.M. Lee, M.L. Papathakis, H.M.C. Feng, G.F. Hunter and J.E. Carr, Fresenius J. Anal. Chem., 339 (1991) 376–383.
[7] J. Fillion, R. Hindle, M. Lacroix and J. Selwyn, J. AOAC Int., 78 (1995) 1252–1266.
[8] P.T. Holland and C.P. Malcom, in T. Cairns and J. Sherma (Editors), Emerging Strategies for Pesticide Analysis, CRC Press, Boca Raton, FL, 1992, Ch. 4, pp. 71–98.
[9] M.L. Hopper and J.W. King, J. Assoc. Off. Anal. Chem., 74 (1991) 661–666.
[10] A. Valverde-García, A.R. Fernandez-Alba, A. Agüera and M. Contreras, J. AOAC Int., 78 (1995) 867–873.
[11] A. Valverde-García, A.R. Fernandez-Alba, M. Contreras and A. Agüera, J. Agric. Food Chem., 44 (1996) 1780–1784.
[12] T. Cairns, K.S. Chiu, D. Navarro and E. Siegmund, Rapid Comm. Mass Spectrom., 7 (1993) 971–988.
[13] N.L. Porter, A.F. Rynaski, E.R. Campbell, M. Saunders, B.E. Richter, J.T. Swanson, R.B. Nielsen and B.J. Murphy, J. Chromatogr. Sci., 30 (1992) 367–373.
[14] P.G. Thompson and L.T. Taylor, J. High Resolut. Chromatogr., 17 (1994) 759–764.
[15] B.W. Wenclawiak, O.P. Heemken, D. Sterzenbach, J. Schipke, N. Theobald and V. Weigelt, Anal. Chem., 67 (1995) 4577–4580.
[16] J.J. Langenfeld, M.D. Burford, S.B. Hawthorne and D.J. Miller, J. Chromatogr., 594 (1992) 297–307.
[17] J. Vejrosta, A. Ansorgová, J. Planeta, D.G. Breen, K.D. Bartle and A.A. Clifford, J. Chromatogr. A, 683 (1994) 407–410.
[18] Y. Yang, S.B. Hawthorne and D.J. Miller, J. Chromatogr. A, 699 (1995) 265–276.
[19] N. Hüsers and J. Kleiböhmer, J. Chromatogr. A, 697 (1995) 107–114.
[20] S. Bøwadt, B. Johansson, F. Pelusio, B.R. Larsen and C. Rovida, J. Chromatogr. A, 662 (1994) 424–433.
[21] L.J. Mulcahey and L.T. Taylor, Anal. Chem., 64 (1992) 2352–2358.
[22] L.J. Mulcahey, J.L. Hedrick and L.T. Taylor, Anal. Chem., 63 (1991) 2225–2232.
[23] M. Ashraf-Khorassani, R.K. Houck and J.M. Levy, J. Chromatogr. Sci., 30 (1992) 361–366.
[24] W.N. Moore and L.T. Taylor, Anal. Chem., 67 (1995) 2030–2036.
[25] D.J. Miller, S.B. Hawthorne and M.E.P. McNally, Anal. Chem., 65 (1993) 1038–1042.
[26] M.L. Hopper, J.W. King, J.H. Johnson, A.A. Serino and R.J. Butler, J. AOAC Int., 78 (1995) 1072–1079.
[27] J.E. France, J.W. King and J.M. Snyder, J. Agric. Food Chem., 39 (1991) 1871–1874.
[28] B. Murugaverl, A. Gharaibeh and K.J. Voorhees, J. Chromatogr. A, 657 (1993) 223–226.
[29] R.J. Maxwell, A.R. Lightfield and A.A.M. Stolker, J. High Resolut. Chromatogr., 18 (1995) 231–234.
[30] R.J. Maxwell, O.W. Parks and E.G. Piotrowski, J. High Resolut. Chromatogr., 15 (1992) 807–811.
[31] C.A. Thompson and D.J. Chesney, J. Chromatogr., 543 (1991) 187–194.
[32] P.R. Eckard and L.T. Taylor, J. High Resolut. Chromatogr., 19 (1996) 117–120.
[33] A. Andersson, pers. comm.
[34] S. Schachterle and C. Feigel, J. Chromatogr. A, 754 (1996) 368.